

CLAIMS

1. An optical assembly comprising a light source, at least one sample vessel and a detector, the at least one vessel being positioned in a light path or paths created between the source and the detector in manner to enable transmission of light through the vessel wherein the light source is adapted to provide a beam of substantially collimated light, the detector comprises a plurality of detector locations and the vessel comprises a wall and core of relative shape and dimensions adapted to contain a sample for detection and to define at least two spatially separated transmitted light paths, a first wall path which enters and exits the vessel walls only, spatially separated from a second core path which enters and exits the vessel walls and additionally the vessel core, wherein the spatially separated wall and core paths are coupled to individual detector locations on the detector, and the detector is an array detector.
2. Optical assembly of Claim 1 wherein the assembly defines a central core path and two peripheral wall paths either side thereof or an annular wall path thereabout.
3. Optical assembly of any of Claims 1 and 2 wherein core and wall path beams are spatially close, preferably adjacent, on the array detector, facilitating direct referencing as the ratio of the core beam to the wall beam.
4. Optical assembly of any of Claims 1 to 3 wherein the light source comprises at least one wavelength of light that is absorbed by one or more absorbing species, the absorbance of which is to be detected.

5. Optical assembly of any of Claims 1 to 4 wherein the light source output is coupled from an optical fibre.
6. Optical assembly of any of Claims 1 to 5 wherein light is of wavelength 5 in the range 160 to 1200 nm, preferably 180 or 190 to 1200 nm, corresponding to UV, UV-vis to near infra red (NIR).
7. Optical assembly of any of Claims 1 to 6 wherein the at least one sample vessel in the assembly of the invention comprises a cell or conduit 10 which may be closed or open ended and closed or open based and topped, intended for static or dynamic sample detection.
8. Optical assembly of any of Claims 1 to 7 wherein the sample vessel is a single cell or one of a plurality of cells in an array; or is a single capillary or 15 one of a plurality of capillaries in a microcapillary array or a microfabricated channel array.
9. Optical assembly of any of Claims 1 to 8 wherein illumination:detection magnification is in the range 0.8 – 1.5:1.

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10. Optical assembly of any of Claims 1 to 9 which is characterised by respective outer and inner diameter of a sample vessel, and by respective refractive indices of vessel walls and of sample, wherein refractive index of the vessel wall is greater than that of the bulk phase of any sample comprised 25 in the core whereby the wall and core paths are spatially separated.
11. Optical assembly of any of Claims 1 to 10 in which the sample vessel comprises an outer and inner wall which are of similar cross-section or shape or different for which a cross-section in a plane including the transmission

light path is independently square or rectangular, curved circular or angular or a combination thereof and is symmetrical or asymmetrical.

12. Optical assembly of any of Claims 1 to 11 wherein outer and inner vessel walls are of coaxial circular cross-section thereby defining an annular wall having outer and inner diameters such that refraction and spatial separation of core and wall beams is achieved.
13. Optical assembly of Claim 12 characterised by i.d.(vessel) in the range 3 micron to 20 mm, o.d.(vessel) in the range 4 micron to 30mm, refractive index (vessel) in the range 1.3 - < 1.6, refractive index (sample) in the range 1.3 to in excess of 1.5, ratio $d/o.d.$ is 0.5 to 10 and d is in the range 20 micron to 300 mm.
14. Optical assembly of any of Claims 1 to 13 wherein a vessel is composed of transparent polymer, glass, quartz, silica, fused silica or other optical grade material.
15. Optical assembly of any of Claims 1 to 14 wherein the vessel wall is optically transparent in part or whole.
16. Optical assembly of any of Claims 1 to 15 wherein the vessel wall is of the same material throughout.
17. Optical assembly of any of Claims 1 to 16 wherein the vessel i.d./o.d. ratio falls in a range giving suitable beam separation with all common solvents.

18. Optical assembly of any of Claims 1 to 16 wherein the vessel i.d./o.d. is given by

$$\frac{\text{i.d.}}{\text{o.d.}} + \text{r.i.}(\text{solvent}) + \text{r.i.}(\text{vessel}) \Rightarrow \frac{d_{\min}}{\text{o.d.}} \Rightarrow d_{\min}$$

5 where + infers a spatial relation which would be calculated using suitable ray tracing software to give values for vessel and assembly dimensions.

19. Optical assembly of any of Claims 13 to 18 wherein $d/\text{o.d.}$ is in the range 0.5 – 5 and d is of the order of 50 – 360 micron.

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20. Optical assembly of any of Claims 13 to 19 wherein d is of the order of 200 micron.

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21. Optical assembly of any of Claims 1 to 20 wherein an array detector comprises a solid state sensing device.

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22. Optical assembly of any of Claims 1 to 21 wherein an array detector comprises a CCD including a surface stud comprising a coating to absorb incident light and reemit at a different wavelength, to convert UV to visible light, to allow detection by the CCD, wherein the coating is applied directly to the stud or to a cover slip interleaved between the stud and vessel, facilitating recoating as needed, by replacing the cover slip without need to replace the stud.

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23. Optical assembly of any of Claims 1 to 22 which comprises means for real-time signal processing for optimum peak detection and parameterisation/characterisation, and means for automatic system

management including closed-loop feedback control of the apparatus and systems.

24. Optical assembly of any of Claims 1 to 23 in which closed-loop
5 feedback control means includes means for stopping or slowing the flow
following initial observation in the detection means to allow sample to reside
in the detector window and give longer times for data acquisition and
enhanced signal to noise or to enable fraction collection, or to direct a fraction
to an analysis means.

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25. Method for detection of light transmitted through at least one sample
contained within the core of at least one sample vessel of an optical assembly
as hereinbefore defined in any of Claims 1 to 24, comprising illuminating the
vessel with a substantially collimated light source or sources and detecting
15 transmitted light in an array detector, wherein transmitted light is spatially
separated into at least two light paths, a wall path which has passed through
the vessel walls only, spatially separated from a core path which has passed
through the walls and core, wherein the spatially separated light beams are
coupled to individual detection locations on the array detector.

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26. Method of any of Claims 25 to 25 which is a method for detecting light
from a static or dynamic sample.

27. Method of any of Claims 25 to 26 wherein a sample is any vessel
25 contents which comprise a single or multiple components, wherein multiple
components are present as a homogeneous or heterogeneous mixture, and
optionally undergo migration with time.

28. Method of Claim 27 wherein a sample is a plurality of liquid phase components optionally including a dissolved phase component; or includes one or a plurality of analytes which it is desired to detect in one or a plurality of solvents or like bulk phase sample component,

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29. Method of Claim 28 wherein a sample includes one or a plurality of analytes which it is desired to detect in the course of a chemical reaction generating or consuming a species as analyte.

10 30. Method of any of Claims 25 to 29 which is a method selected from pressure driven or electrical driven separations column or capillary separations including high pressure liquid chromatography (HPLC) or capillary electrochromatography (CEC), micellar electrokinetic chromatography, and capillary electrophoresis (CE) including the focusing and concentrating
15 techniques of isoelectric focusing (IEF), isotachophoresis (ITP), capillary zone electrophoresis (CZE) and dynamic field gradient focussing (DFGF).

31. Method of any of Claims 25 to 30 in which a sample is of one or more small or large molecules present in liquid or gel phase suitably in solution with
20 liquid phase solvent or cosolvent characterised by a refractive index of lower than but of similar order to that of the sample vessel walls.

32. Method of any of Claims 25 to 31 which additionally comprises selecting a sample for analysis, determining individual wavelengths at which
25 absorption by desired sample components is strongest, checking refractive index of the sample in order to select a suitable sample vessel which when containing the sample and when illuminated will generate spatially separated beams as hereinbefore defined or selecting a suitable combination of optical components and filters and a suitable vessel to detect an array separation to

couple spatially separated beams to independent locations on the detector array.

33. Method of any of Claims 25 to 32 in which sample is introduced into the at least one sample vessel by injection, loop injection, pipette, hydrostatic, or electrokinetic injection and is removed from the vessel by injection, electrospray or interface for discard or to a further vessel for storage or to a down stream identification means.
- 10 34. Method of any of Claims 25 to 33 which comprises imaging the transmitted light detected by the detection means
35. Method of Claim 34 wherein imaging is in the form of a CCD image.
- 15 36. Method of any of Claims 25 to 35 which comprises referencing the light detected by the detection means by means of exposure referencing wherein the ratio of the core beam intensity to the wall beam intensity gives a value for the sample intensity at each location with elimination of excess or flicker noise due to light source fluctuation.
- 20 37. Method of any of Claims 25 to 36 which includes subsequently measuring the amount of absorption of light by species in the sample vessel which indicates the amount of absorbing species by measuring the intensity of light in the absence and presence of the sample, or by measuring intensity of light in a wall beam and a core beam, wherein the logarithm of the ratio, taken in conjunction with values measured in the absence of sample, provides the absorbance according to the Beer-Lambert Law.

38. Module optical assembly of any of Claims 1 to 24 for use with a column or capillary separating device as known in the art, wherein the vessel is a capillary or column comprising interfacing means at one end for inserting into the outlet of a column or capillary separating device or along the length 5 thereof, the capillary or column optionally comprising interfacing means enabling insertion into the inlet of an analysing means at the other end.

39. Clip-on device optical assembly of any of Claims 1 to 24 comprising means for locating about a section of a capillary or column separating device 10 which is of suitable i.d, o.d. and refractive index as hereinbefore defined and is stripped of any surface coating to facilitate the operation of the method of the invention, whereby the stripped capillary or column provides the sample vessel of the assembly.

15 40. Apparatus for chemical reaction or synthesis and analysis or for sample separation or transport wherein the apparatus comprises the optical assembly of any of Claims 1 to 24 as hereinbefore defined in which the chemical reaction vessel itself is cylindrical and the reaction monitored in batch flow mode as a function of time, and feedback control used to halt reaction or in 20 which the reaction vessel is tubular and used in continuous flow mode.

41. Use of the optical assembly, method and apparatus, module or clip-on as hereinbefore defined in any of Claims 1 to 40 in the pharmaceutical, biomedical and bioscience, agrochemical, veterinary and materials fields, for 25 detection, analysis, characterisation and quantification of samples contained in a vessel, and optionally further collecting separated components thereof.

42. Use according to Claim 41 in combinatorial chemistry; in metabolomics, proteomics or genomics, assay and high throughput analysis applications, or for sample separation by chromatography or electrophoresis.

5 43. Use according to Claim 41 or 42 in enzyme assays, high sensitivity analyses, column chromatography, capillary electrophoresis with real time or post separation analysis.

10 44. Assembly, method, module, clip-on device or use thereof substantially as herein described or illustrated in the description, the examples and/or the figures.